# IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| Applicants: | Stavrianopoulos et al. |
| Serial No. | 08/486,070 |
| Filed: | June 7, 1995 |
| Title: | COMPOSITION EMPLOYING CHEMICALLY LABELED OLIGO-NUCLEOTIDE OR POLYNUCLEOTIDE, AND KIT AND APPARATUS CONTAINING SAME |

Group Art Unit: 1634
Former Group Art Unit: 1809
Examiner: Ardin H. Marschel, Ph.D

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Honorable Commissioner of Patents and Trademarks
The United States Patent and Trademark Office
Washington, D.C. 20231

## DECLARATION OF DR. DEAN L. ENGELHARDT IN SUPPORT OF POSSESSION OF CLAIMED SUBJECT MATTER AND NOVELTY OF INVENTION

I, Dean L. Engelhardt, Ph.D, hereby declare as follows:

1. I am currently employed by Enzo Biochem, Inc., 527 Madison Avenue, New York, New York 10022 as Senior Vice President, having held that position since 1988. Prior to my employment at Enzo Biochem, Inc., I was Associate Professor of Microbiology at Columbia University College of Physicians and Surgeons, New York City, having earlier obtained my doctorate from Rockefeller University in New York City. A copy of my curriculum vitae is attached as Exhibit A.

2. In addition to my position as Senior Vice President of Enzo Biochem, Inc., I have also served as Director of Research in which capacity I have overseen scientific research activities for the company and its subsidiaries. I also continue to oversee various research projects. Among my responsibilities at Enzo Biochem, Inc. have been the development of new nucleic acid technology and hybridization formats, including new diagnostic and therapeutic approaches and agents based upon nucleic acid technology. Currently, I am directing research and human clinical trials in connection with Enzo's Investigational New Drug (IND) application that was submitted last year to the Food and Drug Administration (FDA). The IND applications concerns the use of genetic antisense nucleic acid medicine for HIV-1.

3. I am familiar with the contents of this application and portions of its prosecution history. I understand that claims 48-100 and 102-182 represent the presently pending claims in the application, and that these claims are directed variously to a composition of matter, a system, an apparatus and an array. Among the claims are a composition of matter represented by independent claims 48 and 77, a transparent non-porous or translucent non-porous system represented by independent claim 102, an apparatus set forth in independent claims 100 and 132, and an array that is recited in independent claim 141. A copy of the pending claims, 48-100 and 102-182, is attached as Exhibit B.

4. I have read the Office Action dated January 21, 1998 that was issued in connection with this application. I understand that in the January 21, 1998 Office Action the composition of matter claims and system claims represented by claims 60 and 114, respectively, and the composition of matter recited in claims 77-85 and 87-99 were rejected for new matter. That is to say, these claims were rejected as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

A. The Examiner's position on the new matter rejection of claims 77-85 and 87-99 as stated in the January 21, 1998 Office Action is as follows:

> Consideration of the disclosure as filed has failed to reveal the limitation of instant claims 60 and 114 directed to the support and system being composed of different materials. This limitation is therefore NEW MATTER.
>
> Claims 77-85 and 87-99 contain NEW MATTER because the oligonucleotide or polynucleotide is cited as fixed or immobilized to the system rather than being limited to being fixed or immobilized to the solid support within such a system. Consideration of the disclosure as filed has not revealed fixing or immobilizing to a system as now cited in claims 77 etc.

B. The Examiner's position in part on the anticipation rejection as stated in the January 21, 1998 Office Action is as follows:

> Kourilsky et al. disclose the preparation of various probes which hybridize to target nucleic acid on page 3. In particular cytochrome C is utilized to attach biotin to a probe. Beta-galactosidase is linked to the hybridized probe via avidin which is then centrifuged as given on said page 3, lines 42-49. Centrifuge tubes are inherently either glass or plastic. The pellet from this centrifugation is the practice of at least temporarily fixing the probe with enzymatic label onto a solid support device or system. The pellet is resuspended and optically assayed in solution for the enzymatic activity of the beta-galactosidase. This reads on the instant claims.
>
> Applicants argue the below art rejections in that the practice of in-situ hybridization is a very specialized type of methodology and different from the soluble signal generation practice as instantly claimed. In response applicants are reminded that compositions, apparati, and systems are claimed and not methods. Therefore, if a reference meets the composition, apparatus, or system limitations, it anticipates the instant invention even if a number of uses can be practiced for the claimed invention. In other words patentable weight is not given to use limitations if they do not limit the actual composition etc. limitations.

5. It is my opinion that the originally filed specification does indeed support the subject matter of pending claims, 60 and 114, and 77-85 and 87-99, which are adequately described to the point that a skilled artisan would have reasonably concluded that the original disclosure evidenced possession of the invention currently being claimed. It is also my opinion that the pending claims define an invention that contains materially different elements from the Kourilsky document cited in the anticipation rejection. I am making this Declaration to substantiate both that the support and adequate description in the specification for claims 60, 77-85, 87-99 and 114, as well as the novelty of the invention defined by the pending claims.

POSSESSION OF THE CLAIMED SUBJECT MATTER

Claims 60 and 114

6. With respect to the support and description in the specification for the invention recited in claims 60, 77-85, 87-99 and 114, I offer the following remarks. Claims 60 and 114 are directed to specific embodiments for a composition of matter and a transparent non-porous or translucent non-porous system containing a fluid or solution, respectively. Claim 60 recites "[t]he composition according to claim 48, wherein said solid support and said system are composed of different materials." Claim 114 recites "[t]he system according to claim 102, wherein said solid support and said system are composed of different materials." In my opinion, the specification supports and adequately describes the aforementioned embodiments wherein the solid support and the system are composed of different materials. The support and description in the specification would have reasonably conveyed to one skilled in the relevant art to which the present invention pertains that the inventors were in possession of this subject matter at the time the application was filed.

7. The specification discloses that the system for containing or retaining a fluid or solution, be it a product, device, apparatus, wells, tubes, cuvettes, and the like, can be transparent non-porous or translucent non-porous, and that the solid support can be non-porous or porous.

A. On page 14, second paragraph, there is disclosed " . . . a device containing a portion for retaining a fluid." Later in the that paragraph, it is disclosed that "[t]he portion of the device for containing the fluid is desirably a well, a tube, or a cuvette." Also disclosed in the same paragraph is " . . . an apparatus comprising a plurality of such devices for containing a fluid, in which at least one such device contains the above-described immobilized polynucleotide sequence, polynucleotide or oligonucleotide probe, signalling moiety, and soluble signal." On page 13, last paragraph, there is disclosed:

> Yet another aspect of the method of the present invention involves generating the soluble signal from the probe-analyte hybrid in a device capable of transmitting light therethrough for the detection of the signal by spectrophotometric techniques.

B. The specification also discloses that the solid support can be non-porous and transparent as well as conventional porous materials. For example, on page 10, the first full paragraph, it is disclosed:

> . . . In the practices of this invention, it is preferred that the solid support to which the analyte is fixed be non-porous and transparent, such as glass, or alternatively, plastic, polystyrene, polyethylene, dextran, polypropylene and the like. Conventional porous materials, e.g., nitrocellulose filters, although less desirable for practice of the method of the present invention, may also be employed as a support.

C. In the above-quoted portion, the specification plainly indicates that the solid support can be made of non-porous and transparent materials, such as glass and plastic, or conventional porous materials, such as nitrocellulose filters. In other portions, for example, page 14, second paragraph, the specification also indicates that the system, e.g., a product, device, apparatus, wells, tubes, cuvettes, and the like, are in every instance made of non-porous materials for containing or retaining a fluid or solution. Thus, the specification would reasonably convey to one skilled in the relevant art that at the time the application was filed the inventors were in possession of the subject matter of claims 60 and 114 wherein the solid support and the system are made or composed of different materials.

D. My opinion is further bolstered by the claim language in several of the originally filed claims. The following original claims support the language of claims 60 and 114 with respect to the system and the solid support are composed of different materials:

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| claim 5 | (" . . . characterized in that said solid support is non-porous,"); |
| claim 8 | (" . . . characterized in that said solid support is porous."); |
| claim 16 | (" . . . wherein said detecting step further comprises generating said soluble signal in a device capable of transmitting light therethrough for the detection of said soluble signal by |

spectrophotometric techniques.");

claim 17 ( . . . characterized in that said device is selected from the group consisting of a well, a tube, a cuvette and an apparatus which comprises a plurality of said wells, tubes or cuvettes.");

and claim 21 ( . . . wherein said means for containing a fluid is selected from the group consisting of a well, a tube, and a cuvette.").

From a reading of the above-quoted original claims and/or the specification quoted above in Paragraphs 7A and 7B, one skilled in the relevant art would reasonably conclude that the inventors were in possession at the time the application was filed of the claimed subject matter wherein the system and the solid support are composed of different materials.

Claims 77-85 and 87-99

8. It is my position that the specification discloses that the oligonucleotide or polynucleotide is fixed or immobilized to the system and as such, does not limit the present invention to having the oligonucleotide or polynucleotide fixed or immobilized to a solid support within the system. The subject matter of claims 77-85 and 87-99 specifically calls for a composition of matter comprising a transparent non-porous or translucent non-porous system capable of retaining or containing a fluid or solution. The system comprises a double-stranded oligonucleotide or polynucleotide which is directly or indirectly fixed or immobilized to the system wherein one of the strands produces a soluble signal generated or generatable from a chemical label or labels which comprise a signalling moiety or moieties. As set forth in the next paragraph, this subject matter is supported variously in the specification.

A. In the specification, the second paragraph on page 14, there is disclosed:

> A further aspect of the present invention provides products useful in the disclosed method for detection of a polynucleotide sequence. Among these products is a device containing a portion for retaining a fluid. Such portion contains an immobilized polynucleotide

> sequence hybridized to a polynucleotide or oligonucleotide probe. The probe, as described above, has covalently attached thereto a chemical label including a signalling moiety capable of generating a soluble signal. Also part of the device is a soluble signal, preferably a colored or fluorescent product, generatable by means of the signalling moiety. The portion of the device for containing the fluid is desirably a well, a tube, or a cuvette. A related product of the invention is an apparatus comprising a plurality of such devices for containing a fluid, in which at least one such device contains the above-described immobilized polynucleotide sequence, polynucleotide or oligonucleotide probe, signalling moiety, and soluble signal.

That above-quoted disclosure clearly indicates that the double-stranded oligonucleotide or polynucleotide can be fixed or immobilized to the system, be it a product, device, apparatus, wells, tubes, cuvettes, and the like. As such, the specification would reasonably convey to one skilled in the relevant art to which the present invention pertains that the subject matter of claims 77-85 and 86-99 was in the inventors' possession when the application was filed.

B. My opinion expressed in the preceding paragraph is also bolstered by the original claims that were filed. In both originally filed claims 20 and 23, a device and an apparatus are claimed, respectively, in which an immobilized polynucleotide sequence is hybridized to a polynucleotide or oligonucleotide probe, without *any* recitation or reference to a solid support. The text of original claims 20 and 23 follows below:

> 20. A device which comprises:
> means for containing a fluid comprising:
> (i) an immobilized polynucleotide sequence hybridized to a polynucleotide or oligonucleotide probe, said probe having covalently attached thereto a chemical label comprising a signalling moiety capable of generating soluble signal, and
> (ii) a soluble signal generated by means of said signalling moiety.
>
> 23. An apparatus comprising:
> a plurality of means for containing a fluid, wherein at least one of said means comprises:
> (i) an immobilized polynucleotide sequence hybridized to a polynucleotide or oligonucleotide probe, said probe having covalently attached thereto a chemical label comprising a signalling moiety capable of forming a soluble signal, and
> (ii) a soluble signal generated by means of said signalling moiety.

In my opinion, therefore, the originally filed claims, taken alone or with other portions in the specification, for example, page 14, second paragraph, conveys to one skilled in the relevant art that the inventors possessed the subject matter of claims 77-85 and 86-99, wherein an oligonucleotide or polynucleotide is fixed or immobilized to the system as set forth in the claims at hand.

9. On the basis of the foregoing remarks and above-quoted portions from the specification, I conclude that the original disclosure reasonably conveys to one skilled in the relevant art that at the time their application was filed, the inventors were in possession of the subject matter of claims 60 and 114, and 77-85 and 86-99.

NOVELTY OF THE CLAIMED INVENTION

10. Regarding the novelty of the invention being claimed in pending claims 48-100 and 102-182, I offer the following remarks in support of the invention. Each of the pending claims is directed to subject matter in which a soluble signal is generated or generatable from a signaling moiety or moieties of a chemically labeled double-stranded oligonucleotide or polynucleotide which is directly or indirectly fixed or immobilized to a solid support which is in a transparent non-porous or translucent non-porous system (claims 48-76 and 86, 109, 133, 135, 138 and 140, and 102-108, 110-131, 134, 137, 139 and 142), or is directly or indirectly fixed or immobilized directly to such system (claims 77-85, 87-99, 133, 136 and 141). The apparatus of claims 100 and 132 also include the element of a means for producing a soluble signal generated or generatable from such chemical label or labels comprising a signaling moiety or moieties. It is my opinion and conclusion that the Kourilsky document, GB 2,019,408 B, cited against the pending claims, does not disclose the instantly claimed element of a soluble signal generated or generatable from a chemically labeled oligonucleotide or polynucleotide.

11. In further detail, it is my opinion and conclusion that Kourilsky's disclosure does not teach or suggest the present invention at hand because it fails to generate *any* soluble signal where the biotin labeled RNA is hybridized to the mouse DNA and when fixed or immobilized to a solid support. As the Examiner correctly stated in the January 21, 1998 Office Action:

> ". . . The pellet from this centrifugation [Kourilsky et al.] is the practice of at least temporarily fixing the probe with enzymatic label onto a solid support device or system. The pellet is resuspended and optically assayed in solution for the enzymatic activity of the beta-galactosidase. . ."

In fact, Kourilsky's disclosure is fatally flawed by its clear lack of any solid support so much so that they have to subject their sample to drastic ultracentrifugation in order to separate hybridized RNA-DNA from unhybridized nucleic acids. It is only due to centrifugal force that any pellet is formed within the centrifugation tube, and the formation of that pellet does not constitute fixation or immobilization as it is understood in the field of nucleic acid technology or the present invention. Kourilsky's use of ultracentrifugation prevents altogether *any* generation of a soluble signal from the pellet (Kourilsky et al. term it a "culot") which is not fixed or immobilized to any solid support, including the walls of the centrifugation tube. In fact, Kourilsky's disclosure specifically calls for resuspension in order to dislodge the pellet from the centrifugation tube wall. In no sense does pelleting as it occurs during centrifugation constitute fixation or immobilization. In contrast to Kourilsky's disclosure, the present invention provides for a soluble signal that is generate or generatable from a fixed or immobilized chemically labeled oligonucleotide or polynucleotide. Thus, Kourilsky et al. in no way generates or can generate a soluble signal from their biotin-labeled RNA-mouse DNA hybrid that is pelleted by means of ultracentrifugation and resuspended (detached) before assaying for beta-galactosidase activity. It is impraticable if not impossible to generate a soluble signal from Kourilsky's pelleted product. I am not aware of any instance or report where a soluble signal was generated from a pelleted product such as disclosed in Kourilsky's patent.

12. In addition to the lack of a solid support or system for fixing or immobilizing the labeled RNA-DNA hybrid, there are other deficiencies in Kourilsky's disclosure that prevent if not severely limit the generation of a soluble signal. For one, Kourilsky et al. terminally label their RNA with pancreatic ribonuclease. This is disclosed on page 3 of Kourilsky's disclosure:

> Experiments were carried out on the model consisting of detecting the presence of a mouse DNA by hybridization of this DNA with a mouse ribosomic RNA used as a probe.
>
> Mouse DNA (100 μg per 100 μl of aqueous solution) is denatured by addition of soda (10 μl of 1 M NaOH). 10 minutes later, the solution was brought back to pH neutral by the addition of 10 μl of 1.5 M acid sodium phosphate $NaH_2PO_4$.
>
> 1 μg of ribosomic RNA labelled with biotin by means of cytochrome C, prepared by the technique of Manning & Coll., is added to the denatured DNA solution. This volume was adjusted to 160 μl with water. 40 μl of a solution having a concentration of mineral salts equal to twenty times that of the solution called SSC (abbreviation of the English expression "standard saline citrate") and 200 μl of redistilled or deionized formamide was then added to the medium. It is recalled that the SSC solution is an aqueous solution of 0.15 M sodium chloride, 0.015 M sodium citrate, at pH 7.0.
>
> The mixture was incubated until the next day at ordinary temperature, then dialyzed at 4° against a solution having a double concentration of the SSC solution, then for 8 hours against 500 ml of a phosphate buffer at pH 7.0 containing phosphate at a concentration of 0.1 M, sodium chloride at a concentration of 1 M and ethylene-diamine-tetrasodium acetate (EDTA) at a concentration of 0.01 M. The latter dialysis is then repeated twice, each time for 8 hours.
>
> The solution thus-obtained **was treated with *pancreatic ribonuclease*** for 1 hour at ordinary temperature, to obtain a final concentration of 10 μg per ml of ***ribonuclease***, this treatment permitting the degradation of the non-hybridized RNA.
>
> To the medium obtained was then added a solution of cytochrome C (1 mg per ml) and I microliter of a solution containing 1 mg per ml of avidin and 2 mg per ml of β-galactosidase, of which 1 molecule of β-galactosidase in seven is coupled with avidin. It is mixed and the solution is then left to stand at 4°C for 4 hours. The medium was then diluted to 10 ml with the phosphate dialysis buffer and the solution obtained is subjected to ultracentrifugation for 1 hour at 35,000 rpm (in a BECKMAN ROTOR SW 4.1 centrifuge) "BECKMAN" is a registered Trade Mark. The DNA and the hybridized RNA are to be found in the centrifugation culot, as well as the avidin-β-galactosidase bound to the RNA. The supernatant liquor contains the non-hybridized RNA degraded by the ribonuclease and the unbound avidin β-galactosidase. [emphasis added]

There is sufficient breathing at the ends of any hybridized RNA-DNA that the ribonuclease can cut off the biotin label, thereby preventing detection of the hybrid.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issued thereon.

July 21, 1998
Date

Dean L. Engelhardt

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